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Research Paper

Disulfide-functionalized hyperbranched poly(amidoamine) derivatives as both reductant and stabilizer for the synthesis of fluorescent gold nanoclusters

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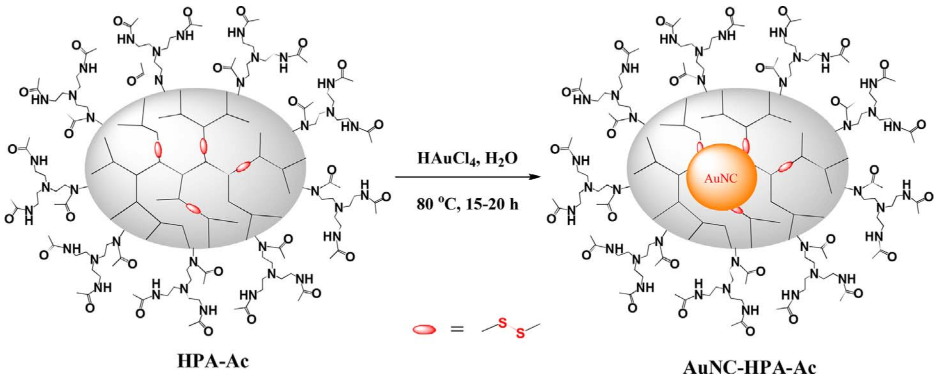
GRAPHICAL ABSTRACT



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ABSTRACT

Hyperbranched polymers were successfully used as reductant and stabilizer for the synthesis of stable water-soluble fluorescent gold nanoclusters (AuNCs) through a one-step bottom-up strategy. The hyperbranched polymers having such performance were acetamide terminated hyperbranched poly(amidoamines) (HPA-Acs) with disulfide functional groups. Small sized (less than 2 nm) AuNCs were formed under the optimal conditions of 80 °C, weak acidic condition (around pH 4.3) for 15–20 h. Through adjusting the feed ratio of $HAuCl_4$ to HPA-Ac, the emitting light could be tuned from yellow to near-infrared. The measurements of UV–vis spectroscopy, transmission electron microscopy and X-ray photoelectron spectroscopy further verified the formation of AuNCs. The quantum yields of the yellow and near-infrared fluorescent AuNCs were ca. 3.6% and 1.2%, respectively. The as-prepared AuNCs showed an excellent stability in a wide pH range (pH 2–12) and possessed low cytotoxicity for cancer HeLa cell, making it a suitable candidate for bioimaging.

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1. Introduction

Gold nanoclusters (AuNCs) typically consist of several to tens of atoms. They are generally less than 1 nm and this size regime is comparable to the Fermi wavelength of the conduction electrons, resulting in molecule-like properties including discreet electronic states and size-tunable fluorescence [1]. Different sized AuNCs exhibit different emissions, which is their main difference from gold nanoparticles [2]. In contrast to semiconductor quantum dots, which are larger in dimensions (~3–100 nm) and typically contain toxic metal species, AuNCs have attracted more attention in bioanalysis and bioimaging applications because of their finer size and low toxicity [3–7].

To date, two major strategies have been developed to synthesize fluorescent AuNCs. One is a top-down strategy by etching large gold nanoparticles with thiols [8–13], biomolecules [14], or multivalent polymers [15]. This strategy usually involves a multi-step process that makes the preparation more complicated and time-consuming. The other is a bottom-up strategy via a one-step reduction of gold salt by reducing agent in the presence of a suitable stabilizer. The frequently-used reductant is sodium borohydride ($NaBH_4$) [16–21]. Because AuNCs are prone to agglomerate into larger clusters or even nanoparticles leading to low quantum yield of fluorescence, stabilizers matching with reductants have been exploited to form an external ligand shell around metal core and enhance resistance to agglomeration and deactivation. Various kinds of stabilizers including synthetic linear polymers [21–24], thiolated peptides [16–18], adenine and its derivatives [25], purine [26], histidine [27–29], diphospine [30], dihydrolipoic acid [31], D-penicillamine [32], alkanethiol [19,20], poly (amidoamine) dendrimer (PAMAM) [33–36], and DNA[37] have been intensively investigated. Furthermore, natural proteins rich in tyrosine and cysteine have been found to act as both reductant and stabilizer in the synthesis of stable AuNCs [38–49], which further simplified the synthesis of AuNCs. The small molecule, histidine, has been also used as reductant and stabilizer in the synthesis of AuNCs, but the obtained AuNCs were not stable and precipitated after storage for 1 or 2 days [29].

As template and stabilizer, dendrimers and hyperbranched polymers are outstanding candidates because of their compact structure and chemical versatility and they have been widely used to prepare nanoparticles [50]. But there are relatively few studies devoted to preparing AuNCs using dendrimers or hyperbranched polymers as stabilizer. Through the bottom-up strategy, only the well-defined dendrimer, PAMAM, has been addressed to prepare AuNCs successfully with the extra addition of reducing agents [33–36]. Duan and Nie adopted a top-down strategy to obtain AuNCs with blue emission through etching gold nanoparticles by hyperbranched polyethylenimine [15]. The procedures for the syntheses of structure-perfect dendrimers are usually multistep and time-consuming, which currently limits their practical use range. For that reason their imperfect analogues, hyperbranched polymers, easily and economically prepared in a one step process have gained increasing interest, especially in industrial applications. Therefore, it is preferable to prepare AuNCs using the cheap and easily scaled-up hyperbranched polymers, instead of the expensive dendrimers. In the present work, yellow and/or near-infrared-emitting fluorescent AuNCs were prepared successfully by employing disulfide-functionalized hyperbranched poly(amidoamine) (HPA) derivatives as both reductant and stabilizer. Many kinds of hyperbranched polymers have been employed to prepare the non-fluorescent Au nanoparticles [50], however, to the best of our knowledge, no hyperbranched polymers have been claimed to be capable of preparing fluorescent AuNCs using the bottom-up strategy so far. Our results reveal that the utilization of HPA derivatives for preparing AuNCs through the bottom-up strategy has the following characters: (1) HPA derivatives can be prepared through two steps and their syntheses can be scaled up easily, which guarantees their cheaper price than dendrimers; (2) Unlike dendrimers [33–36], no extra reducing agents are required for the synthesis of AuNCs; (3) Compared with the hyperbranched polyethylenimine stabilized blue-emitting AuNCs [15], AuNCs prepared from HPA derivatives can emit yellow and/or near-infrared fluorescence, which makes the obtained AuNCs more suitable use in the bio-related fields; (4) The as-prepared AuNCs show an excellent stability in a pH range of 2–12, much wider than the pH stability range of 6–8 exhibited by PAMAM dendrimer stabilized AuNCs [36]. Furthermore, it should be noted that this pH stability range is also wider than that of protein-stabilized AuNCs [51].

2. Experimental

2.1. Materials

Cystamine dihydrochloride (96%) was obtained from Tianjin Heowns Biochem Company and used directly. Acrylyl chloride (99%) and $HAuCl_4{\cdot}4H_2O$ (98%) were purchased from Beijing Ouhe Technology Company. Tris (2-aminoethyl) amine (TAEA, 97%) was obtained from Alfa Aesar and was distilled under reduced pressure before use. Triethyl amine (TEA, A. R.) was purchased from Tianjin University Kewei Chemical Company and distilled before use. 1,6-Hexanediamine (A. R.) was purchased from Tianjin Kemiou Chemical Reagent Company. Acetic anhydride (Ac_2O, A. R.), sodium hydroxide (NaOH, A. R.), methanol (A. R.), and dimethyl sulfoxide (DMSO, A. R.) were obtained from Tianjin University Kewei Chemical Company. High glucose Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate buffer saline (DPBS), trypsin, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT, 98%), and any other reagents and solvents were purchased from Beijing Solarbio Science & Technology Co., Ltd. *N,N′*-cystaminebisacrylamide (CBA) and *N,N′*-hexmethylenebisacrylamide (HMBA) were synthesized according to the literature [52]. Dithiothreitol (DTT, 99%) was purchased from Shanghai Aladdin biological technology Company and used directly. A series of hyperbranched poly(amidoamine) (HPA) containing different amount of disulfide groups were synthesized by Michael addition reaction between TAEA and different molar ratio of HMBA/CBA according to our previous publication [53].

2.2. Syntheses of acetylated HPA (HPA-Ac)

Under N_2 atmosphere, acetic anhydride (0.25 g, 2.4 mmol) was added dropwise to the mixture of HPA hydrochloride (0.6 g) and triethylamine (0.51 g, 5.0 mmol) dissolved in 5 mL DMSO at 0 °C with vigorous stirring. After a reaction carried out at room temperature for 24 h, the precipitated triethylamine hydrochloride was filtered off and the polymer was then purified by dialysis against water using a benzoylated cellulose membrane (MWCO 3, 500 Da) for 2 days. The product was obtained after lyophilizing.

2.3. Preparation of AuNCs

All glassware was washed with *Aqua Regia* ($HCl{:}HNO_3$ volume ratio = 3:1), and rinsed with ethanol and ultrapure water. In a typical experiment, the pH of the aqueous solution of $HAuCl_4$ was adjusted to be ca. 5.2 with the aqueous solution of NaOH and the final concentration of $HAuCl_4$ was 20 mM. A different amount of the as-prepared $HAuCl_4$ solution were mixed with 10 mL of the aqueous solution of HPA-Ac (5 mg/mL), di-distilled water was added to keep the total volume of mixture at 20 mL, and the pH values of the mixtures were around 4.3, and then the mixture was heated at 80 °C under vigorous stirring for 15–20 h.

2.4. Characterization

^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer. The chemical shifts are given in parts per million (ppm). UV–vis spectra